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HELEN UPDEGRAFF

A DISSERTATION SUBMITTED IN PARTIAL FULFILLMENT OF THE REQUIRE-
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A QUANTITATIVE STUDY OF SOME ORGANIC CONSTITUENTS OF THE SALIVA.*

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Quantitative studies of the saliva have, in the past, been concerned chiefly with the thiocyanate content and the reaction toward indicators. Comparatively few reports of investigations of the organic constituents are to be found and, of these, very few furnish any quantitative data. This is hardly surprising when one considers that, until the development of the microchemical methods which has proceeded so rapidly in the last decade, the difficulties involved in such a study were well nigh insurmountable.

The first precise quantitative studies of the urea content of the saliva were made by Hench and Aldrich (1) and by Schmitz (2). These investigations were concerned chiefly with the pathological variations of the urea content of the saliva in connection with diagnosis of renal disorders, rather than the physiological variations. This latter phase has been studied by Morris and Jersey (3) in the laboratory of the former who has also recently extended his findings to the study of saliva in disease (4). These studies all agree in demonstrating that the sum of the salivary urea and ammonia nitrogen runs roughly parallel to the blood urea nitrogen, but that it is somewhat less.

One of the earliest studies of the ammonia content of saliva and a most careful investigation which appears to have been overlooked or ignored by subsequent workers, was conducted in 1881 by Heyward (5) in the laboratory of Mallet of the University of Virginia. The figures for salivary am-

*A preliminary report of this work was reported in the Proceedings of the Society for Experimental Biology and Medicine (Lewis, H. B., and Updegraff, H., *Proc. Soc. Exp. Biol. and Med.*, 1922-23, xx, 168).

†An abstract of a thesis submitted by Helen Updegraff in partial fulfillment of the requirements for the degree of Doctor of Philosophy in the Graduate School of the University of Michigan.

monia reported, 4.0 to 10.0 mg. per 100 cc. of saliva, agree very closely with the figures found by recent investigators (1, 2, 3). Hench and Aldrich (1) and Schmitz (2) reported figures for salivary urea and ammonia in normal and pathological individuals. They have found that the sum of the urea and ammonia nitrogen of the saliva closely approximates the urea nitrogen of the blood. Moreover, they have observed that, in urea retention, the sum of the urea and ammonia nitrogen of the saliva always increases with an increase in the blood urea nitrogen. They conclude, therefore, that the sum of the urea and ammonia nitrogen of saliva is a valuable index of renal functional capacity. It should be mentioned in this connection that Schmitz satisfied himself, by a series of careful experiments, that the salivary ammonia owes its origin to bacterial action upon the urea normally present in the saliva.

Earlier analyses of the uric acid content of the saliva (6, 7, 8) are open to criticism because of the methods employed. In 1919, Lowenstein and Gies (9) found an average uric acid content of 2.10 mg. per 100 cc. for men, and for women, 1.11 mg. per 100 cc. They also succeeded in separating uric acid in crystalline form from saliva. Morris and Jersey (3) obtained values for uric acid similar to those of Lowenstein and Gies in salivas collected after the chewing of paraffin. They believed, however, that the saliva obtained as the natural secretion of resting glands was much more uniform in its content of uric acid and that a correct picture of salivary secretion was not obtained after paraffin chewing. No reliable figures for the non-protein nitrogen of the saliva are available. The amino acid and creatinine content of saliva have been studied by Morris and Jersey (3).

The progress in the knowledge of the organic constituents of the saliva that has been made indicates the need for more comparative data on the organic constituents of blood and saliva. In obtaining such comparative data it is desirable to use, as far as possible, the same methods for saliva as for blood. We have studied the comparative composition of the saliva and blood of normal individuals with reference to the non-protein nitrogen, urea, ammonia,¹ and uric acid. In all cases an attempt has been made to utilize the standard Folin-Wu procedure for blood or to so modify it as to make it of value for the analysis of saliva. Observations which confirm and extend the work of other investigators as to the origin and significance of the ammonia of the saliva have also been made. Certain differences in the amount of the "undetermined non-protein nitrogen" in the blood and in saliva are considered.

¹ It should be mentioned that no determinations of blood ammonia were made in this investigation. Even under pathological conditions the ammonia of the blood (10) amounts to only a few hundredths of a milligram per 100 cc., an amount which is negligible for all practical purposes.

EXPERIMENTAL.

General Procedure.

The subjects of the experiments were healthy men and women, students or laboratory workers for the most part. The sample was collected from 1 to 1½ hours after a light breakfast. Each subject was first required to rinse the mouth thoroughly with water. Paraffin was chewed and the first few cubic centimeters of saliva were rejected. From 30 to 35 cc. of saliva were then collected, the time required for collection of the sample was noted, and the analysis carried out immediately. When comparative studies were made on the blood, 5 to 10 cc. of blood were drawn from an arm vein. The blood was always drawn immediately upon the conclusion of the period during which the saliva was collected.

Precipitation of Protein.

Before a workable scheme of analysis could be devised for the various organic constituents of saliva, it was desirable to find a simple efficient method for the removal of the protein, comparable to the admirable method of Folin and Wu for blood. A few preliminary trials showed that the Folin-Wu method, as it is applied to blood, is not adapted to the removal of protein from saliva. The filtrations were slow and tedious and the filtrates thus obtained were turbid and unsuited for the various determinations. In the search for a suitable method, all the ordinary protein precipitants were studied. The only combination that effected satisfactory and complete removal of the protein was that of trichloroacetic acid and kaolin. This was, however, open to the objection that the trichloroacetic acid filtrate can be used for the determination of comparatively few constituents.

It seemed, therefore, worth while for a time to persist in the effort to apply the precipitation method of Folin and Wu to saliva. It was found that the salivary protein could be completely precipitated by tungstic acid in the presence of a very small amount of calcium chloride, followed by treatment with kaolin. A further difficulty presented itself when it was attempted to use this filtrate for the determination of uric acid by the recent method of Benedict (11). When the filtrate was made alkaline with sodium cyanide, the solution became quite turbid, due probably

to the precipitation of calcium salts, and the cloudiness was not dissipated upon addition of the arsenophosphotungstic acid reagent. It was then decided to remove the excess calcium by precipitation with a slight excess of oxalic acid. However, since, after addition of the oxalic acid, the pH of the solution was much too low for complete precipitation of calcium as the oxalate (12), it was necessary to correct the pH, by addition of alkali, to the range 4.0 to 5.6. By the use of brom-phenol blue, an indicator efficient within this range of pH, the amount of alkali necessary to raise the pH to the desired value was determined. After the addition of the proper amount of alkali, the filtrate obtained was found to be entirely suitable for the determination of uric acid by the method of Benedict, as well as for the other determinations made.

The method of precipitation which has been found to yield a filtrate adapted to the largest number of determinations is as follows:

To 25 cc. of saliva in a 150 cc. Erlenmeyer flask are added 11.6 cc. of water, 2.5 cc. of 10 per cent sodium tungstate, and slowly, while shaking, 2.5 cc. of 0.66 *N* sulfuric acid. 2 cc. of 10 per cent calcium chloride are then added and the mixture is shaken; 5 cc. of 5 per cent oxalic acid and lastly 1.4 cc. of 10 per cent sodium hydroxide are then added, making a total volume of 50 cc. A rubber stopper is inserted in the mouth of the flask and the mixture is well shaken and allowed to stand for from 5 to 10 minutes. About 2 gm. of kaolin are added and the flask is shaken vigorously a few times. The mixture is poured upon a pleated hardened filter (Schleicher and Schüll, No. 575 is of satisfactory quality) and the funnel is covered with a watch-glass during the filtration which usually proceeds rapidly. The filtrates are often water-clear from the first drop, but should the first 5 or 10 cc. of filtrate be turbid, they should be returned to the filter. The method of precipitation is quite elastic in that much less saliva may be used and the amount of water correspondingly increased. In fact, this is often an advantage as when less saliva is used the speed of filtration is increased. Occasionally one encounters a saliva that filters very slowly and it will then be necessary to use a higher dilution. However, higher dilutions are to be avoided, as a rule, as some of the constituents (*e.g.* uric acid and ammonia) are often present in such minute quantities that accurate determinations cannot be made when the saliva is diluted much more than 1:1

The filtrates obtained by this method are faintly acid to litmus, do not give the biuret test, and are, apparently, entirely free from protein. On standing for 24 hours at room temperature the value

for ammonia in such filtrates remains unchanged, indicating that, once the protein is removed from saliva by this method, no further hydrolysis of the urea occurs. Moreover, the value for uric acid in these filtrates does not change appreciably on standing 24 hours at room temperature. In three experiments the uric acid values immediately after precipitation of the protein were 1.3, 1.1, and 1.1 mg. per 100 cc. After the filtrates had stood 24 hours at room temperature the values were 1.0, 1.03, and 1.0 mg., respectively, variations within the experimental error of the method.

Methods of Analysis.

The protein-free filtrates were analyzed as follows: non-protein nitrogen and glucose, by the method of Folin and Wu; urea and ammonia by the aeration urease method of Folin; and uric acid by the recent modification of Benedict (11). The recovery of pure substances added to the saliva was satisfactory in every case.

In view of the fact that most of the ammonia and urea determinations on saliva reported by others (1, 2) have been conducted upon the secretion without subjecting it to any preliminary treatment, it was deemed advisable to compare the values obtained for urea and ammonia nitrogen in the filtered saliva with those obtained in aliquots of the modified Folin-Wu filtrate from the same sample. The values obtained from the filtered saliva agreed with those obtained from the Folin-Wu filtrate practically within the experimental error of the method as borne out by the following typical data: ammonia nitrogen, 2.6 and 2.7 mg., ammonia plus urea nitrogen, 7.1 and 6.8 mg., urea nitrogen 4.5 and 4.1 mg., per 100 cc. in the Folin-Wu filtrate and untreated saliva, respectively.

DISCUSSION.

When it was satisfactorily demonstrated that the various organic constituents, mentioned above, could be accurately determined in the saliva by the technique just outlined, a large number of normal salivas were analyzed in order to gain some idea of the range of normal values. In most of the salivas examined the total solids were determined to find whether there was any evident relation between solids and the amount of any of the organic constituents.

No such relation could be established, however. In Table I is presented a summary of all the salivary analyses made in this investigation with the range through which the amounts of each constituent varied.

The results of comparative studies of the blood and saliva are presented in Tables II and III. The data in Table III include

TABLE I.
Summary of Analyses of Saliva.

The figures represent milligrams per 100 cc. of saliva.

Constituent.	No. of sub-jects.	No. of speci-mens.	Range.	Aver-age.
Men.				
			mg.	mg.
Total solids.....	52	66	386.0-840.0	597.0
Non-protein nitrogen.....	50	62	7.0- 26.7	13.5
Ammonia nitrogen.....	27	31	2.1- 13.2	6.1
" plus urea nitrogen.....	14	18	8.5- 15.9	11.5
Urea nitrogen.....	14	16	0.0- 6.7	4.2
Uric acid.....	55	72	0.5- 2.9	1.7
Women.				
Total solids.....	17	27	406.0-860.0	544.0
Non-protein nitrogen.....	17	19	5.6- 24.4	11.6
Ammonia nitrogen.....	7	7	2.8- 8.3	4.6
Uric acid.....	17	27	0.6- 2.4	1.5
Totals—men and women.				
Total solids.....	69	93	386.0-860.0	581.0
Non-protein nitrogen.....	67	81	5.6- 26.7	13.0
Ammonia nitrogen.....	34	38	2.1- 13.2	5.7
" plus urea nitrogen.....	15	19	6.8- 15.9	11.3
Urea nitrogen.....	15	17	0.0- 6.7	4.1
Uric acid.....	72	99	0.5- 2.9	1.6

also urea, the ammonia plus urea nitrogen, and the residual non-protein nitrogen of the saliva and blood; *i.e.*, the nitrogen not present as urea, ammonia, or uric acid. An inspection of these tables shows that the values for the various organic constituents of saliva are considerably lower than the corresponding values for the blood. This difference is most marked in the case of the non-

TABLE II.

Determination of Total Solids, Ammonia Nitrogen, Non-Protein Nitrogen, and Uric Acid in the Saliva of Fifteen Normal Men and Seven Normal Women and Simultaneous Determinations of Non-Protein Nitrogen, Uric Acid, and Sugar in the Blood of Thirteen Men and Five Women.

The figures represent milligrams per 100 cc. of saliva and blood.

Subject.	Total solids.	Ammonia nitrogen.	Non-protein nitrogen.		Uric acid.		Sugar.	
	Saliva.	Saliva.	Saliva.	Blood.	Saliva.	Blood.	Saliva.	Blood.
Men.								
	mg.	mg.	mg.	mg.	mg.	mg.	mg.	mg.
W. G.	734	7.1	18.6	37.2	1.7	3.2	None.	80
G. H.	570	4.0	11.1	37.3	1.8	4.1	"	93
M. T.	668	5.9	14.3	42.1	1.3	4.4	"	87
R. J.	602	3.1	13.9	35.9	2.0	4.3	"	81
D. M.	496	4.2	14.7	34.1	1.4	4.1	"	83
E. W.	644	2.8	10.2	33.4	2.2	4.3	"	78
C. W.	724	4.4	17.0	36.6	1.7	3.0	"	76
H. D.	564	2.3	10.5	35.9	1.7	3.5	Slight reduction.	84
L. H.	722	5.6	10.0	31.4	1.3	3.9	None.	89
D. D.	704	8.1	15.0		1.7		Slight reduction.	
R. C.	536	3.5	15.0		1.8		None.	
J. B.	506	7.3	13.1	39.4	0.8	3.3	"	92
H. L.	522	7.0	12.0	33.3	2.1	4.1	"	95
W. B.	386	4.0	11.5	35.1	0.7	3.4	Very slight reduction.	80
P. S.	442	2.1	10.9	35.2	0.9	2.6	None.	70
Women.								
W. W.	476	5.0	10.7	32.0	1.1	2.9	None.	86
E. S.	578	3.8	9.8	31.4	1.2	2.8	"	81
S. L.	506	4.6	9.3	32.7	1.2	2.8	"	91
E. T.	412	3.8	8.3		0.6		"	
M. W.	412	2.8	9.6	33.3	2.0	4.6	"	106
M. M.	508	8.3	15.5		1.0		"	
H. U.	490		10.3	28.0	1.5	4.1	"	106

protein nitrogen and uric acid and least in the case of the urea-ammonia nitrogen fraction. In order to indicate more definitely the quantitative relationship between the individual constituents of these two fluids, the values for the saliva in Table III have been

TABLE III.

Determinations of Non-Protein Nitrogen, Ammonia Nitrogen, Urea Nitrogen, Uric Acid, and Residual Non-Protein Nitrogen in the Saliva and Simultaneous Determinations of Non-Protein Nitrogen, Urea Nitrogen, Uric Acid, and Residual Non-Protein Nitrogen in the Blood of Fifteen Normal Individuals.

The figures represent milligrams per 100 cc. of saliva and blood.

Subject.	Non-protein nitrogen.		Ammonia nitrogen.	Ammonia plus urea nitrogen.	Urea nitrogen.		Uric acid.		Residual non-protein nitrogen.			
	Saliva.	Blood.	Saliva.	Saliva.	Saliva.	Blood.	Saliva.	Blood.	Saliva.		Blood.	
	mg.	mg.	mg.	mg.	mg.	mg.	mg.	mg.	mg.	per cent*	mg.	per cent*
J. J.	15.8	37.0		13.5		15.0	0.5	3.2	2.1	13.5	20.9	56.6
	10.8	40.5		10.4		14.4	1.5	3.6	-0.1	-0.9	24.9	61.5
	14.8	42.1	13.2	13.2	0.0	16.7	0.9	3.8	1.3	8.8	24.1	57.3
	11.7	37.4	5.9	10.4	4.5	15.9	1.3	3.9	0.9	7.4	20.2	54.0
P. B.	14.6	37.0	6.9	12.2	5.3	14.6	1.7	3.2	1.8	12.5	21.3	57.7
G. P.	14.7	39.9	9.9	13.6	3.7	14.7	1.7	3.5	0.5	3.6	24.0	60.2
R. C.	11.0	34.8	4.0	10.0	6.0	15.6	2.5	4.4	0.2	1.6	17.7	51.0
C. P.	11.4	35.4	6.4	10.2	3.8	14.9	1.6	3.2	0.7	6.1	19.4	54.9
P. J.	15.8	35.6	5.7	11.1	5.4	16.7	1.8	3.5	4.1	26.0	17.7	49.8
T. T.	20.3	37.5	7.5	8.5	1.0	12.5	1.6	4.8	11.3	55.5†	23.4	62.4
J. B. U.	13.4	32.2	6.6	8.8	2.2	10.2	1.1	3.2	4.2	31.6	20.9	65.0
L. F.	19.6	42.3	10.8	15.9	5.1	21.8	1.4	4.1	3.2	16.5‡	19.1	45.2
A. C.	13.2	35.3	5.8	9.9	4.1	15.2	1.2	2.6	2.9	22.0	19.2	54.5
G. L.	13.8	37.3	6.0	11.9	5.9	18.9	0.9	3.3	1.6	11.6	17.3	46.4
M. L. §	8.2	25.3	4.2	6.8	2.6	9.6	1.0	2.8	1.1	13.2	14.8	58.4
H. E.	19.1	33.9	9.0	13.5	4.5	13.0	1.2	4.1	5.2	27.2	19.5	57.6
	18.8	40.8	5.3	12.0	6.7	14.7	1.8	4.0	6.2	33.0	24.8	60.7
H. L.	12.2	36.7	7.3	11.5	4.2	14.5	1.8	4.0	0.1	0.8	20.9	56.9
D. Mc.	12.0	29.9	5.7	10.9	5.2	14.8	2.7	2.9	0.2	1.7	14.1	47.3

*That is, per cent of non-protein nitrogen.

†Saliva contaminated with a little blood. Note high non-protein nitrogen.

‡Saliva contained some food particles. Note high non-protein nitrogen.

§Woman.

recalculated in terms of the percentage of the corresponding constituent of the blood of the same individual and are recorded in Table IV. In addition, all the comparative figures obtained for blood and saliva have been summarized in Table V.

68 of the salivas analyzed were tested for sugar by the Folin-Wu method. In Table II where figures for blood sugar are recorded, the reaction of the corresponding salivary filtrate to the Folin-Wu reagent is also recorded. A decided reduction was noted in a few of the 68 salivas examined, but the color developed was never of sufficient intensity to permit a quantitative estima-

TABLE IV.

Figures for Saliva from Table III Expressed as Percentage of the Corresponding Constituent in the Blood of the Same Individual.

Subject.	Non-protein nitrogen.	Ammonia plus urea nitrogen as percentage of blood urea nitrogen.	Urea nitrogen.	Uric acid.	Residual non-protein nitrogen.
	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>
J. J.	42.7	90.0		15.6	10.1
	26.7	72.2		41.7	0.0
	35.2	79.0	0.0	23.7	5.4
	31.3	65.4	28.3	33.3	4.5
P. B.	39.5	83.6	36.3	53.1	8.5
G. P.	36.8	92.5	25.2	48.6	2.1
R. C.	31.6	64.1	38.5	56.8	1.1
C. P.	32.2	68.5	25.5	50.0	3.6
P. J.	44.4	66.5	32.3	51.4	23.2
T. T.	54.1	68.0	8.0	33.3	48.3
J. B. U.	41.6	86.3	21.6	34.4	20.1
L. F.	46.3	72.9	23.4	34.2	16.8
A. C.	37.4	65.1	27.0	46.2	15.1
G. L.	37.0	63.0	31.2	27.3	9.3
M. L.*	32.4	70.8	27.1	35.7	7.4
H. E.	56.3	103.9	34.6	29.3	26.7
	46.1	81.6	45.6	45.0	25.0
H. L.	33.2	79.3	29.0	45.0	0.5
D. Mc.	40.1	73.7	35.1	93.1	1.4

*Woman.

tion of the amount of reducing substance present. Nevertheless, in these cases the color developed was deeper than that developed in a blank control containing only the reagents. In the case of three normal individuals the administration of from 100 to 300 gm. of glucose produced a decided hyperglycemia, but no increased amount of glucose was eliminated in the saliva 1 hour after the glucose administration. In view of these results and those of

numerous other investigators, it is probably safe to conclude that glucose, in significant amounts, is not a normal constituent of saliva.

The average normal figures for salivary non-protein nitrogen give, it is believed, an accurate idea of the actual average normal value for this constituent. However, the maximum values (23.7 mg. per 100 cc. for men and 24.4 mg. per 100 cc. for women) obtained for non-protein nitrogen are probably really higher than the actual normal. Very few values were found in the neighborhood of these maximum values and, invariably, when high figures

TABLE V.
Summary of the Comparative Data on Blood and Saliva.

Constituent.	No. of subject.	No. of specimens	Blood.		Saliva.		Saliva.*	
			Range.	Average.	Range.	Average.	Range.	Average.
			mg.	mg.	mg.	mg.	per cent	per cent
Non-protein nitrogen.	31	37	25.3-42.3	35.5	8.2-20.3	13.2	26.7- 56.3	37.0
Ammonia plus urea nitrogen.....	15	19	9.6-21.8	14.9	6.8-15.9	11.3	63.0-103.9	76.1
Uric acid.....	31	41	2.6- 4.8	3.6	0.5- 2.7	1.5	15.6- 93.1	40.1
Residual non-protein nitrogen	15	19	14.1-24.9	20.2	0.0-11.3†	2.5	0.0- 48.3	12.1

*In these two columns the figures for saliva are expressed as percentages of the corresponding constituent in the blood of the same individual.

†Compare foot-note 2 to text.

were obtained, there had been opportunity for some decomposition in the sample; *e.g.*, standing in a warm room for an hour or so before it was possible to precipitate the protein. The very low figure (5.6 mg.) was obtained in only one case and may possibly be ascribed to an experimental error.

It will be observed that the values for ammonia nitrogen show a wide range of variation probably depending upon the extent of bacterial decomposition of salivary urea. The fact that the values for urea nitrogen also showed a similar range of variation is in accord with this idea. The urea nitrogen may even be reduced to zero. Undoubtedly this value also depends upon the extent of

bacterial decomposition that has taken place. This matter will be discussed later in more detail. As already pointed out by Hench and Aldrich (1) and Schmitz (2), the value for ammonia plus urea nitrogen maintains a much greater constancy than the urea nitrogen and is, therefore, to be regarded as more significant than the value for either urea or ammonia nitrogen alone.

The value for uric acid in the saliva varied from 0.5 to 2.9 mg. with an average of 1.6 mg. per 100 cc. It is believed that this figure is an accurate estimate of the average normal value. Very few samples were found to run as high as 2.5 mg. per 100 cc. and the low figure, 0.5 mg., was obtained in only one instance. A study of the salivary uric acid of one normal woman over a period of several weeks indicated that a relatively constant level was maintained in the secretion. The extreme values were 0.9 and 1.7 mg. per 100 cc. with an average value of 1.2 mg. and a maximum deviation from the mean of 0.5 mg.

In agreement with Schmitz we found that the figure for the sum of the urea and ammonia nitrogen approached quite closely the value for the urea nitrogen of the corresponding blood (Table IV). However, in the fifteen individuals in which simultaneous analyses were made of the blood and saliva, the sum of the urea and ammonia nitrogen of the saliva averaged 76.1 per cent of the urea nitrogen of the blood, a value somewhat lower than the average values, 89.4 per cent, reported by Schmitz (2) and 80 per cent by Hench and Aldrich (1). In this connection it is of interest that Myers and Fine (13) have found that the urea of spinal fluid amounts to 88 per cent of that of the blood. In this respect, saliva may be said to resemble spinal fluid.

A consideration of the total average values for the various organic constituents of saliva (Table I) brings out another interesting point which merits discussion here. The average figure for salivary non-protein nitrogen is 13.0 mg. per 100 cc. and for salivary urea plus ammonia nitrogen, 11.3 mg. per 100 cc. Thus in saliva the urea plus ammonia nitrogen comprises about 86.9 per cent of the non-protein nitrogen. This is in marked contrast to blood in which, as a rule, the urea nitrogen amounts to less than 50 per cent of the non-protein nitrogen. Moreover, the average value for salivary uric acid is 1.6 mg. per 100 cc. or, expressed in terms of uric acid nitrogen, 0.53 mg. per 100 cc. Thus the nitro-

gen of the urea, ammonia, and uric acid amounts to 11.83 mg., or 91 per cent of the non-protein nitrogen. There remains only 9 per cent or about 1 mg. unaccounted for.² It is difficult to reconcile these results with the figures for salivary amino acid nitrogen reported by Morris and Jersey (3). These investigators found from 4 to 11 mg. and, as a rule, about 7 or 8 mg. of amino acid nitrogen per 100 cc. of saliva, while our results would indicate that there can be no significant amounts of amino acid nitrogen in this secretion. Saliva thus appears to resemble urine more than blood in that the urea plus ammonia nitrogen accounts for the greater part of the non-protein nitrogen.

The failure to obtain evidence of any significant amount of nitrogen not accounted for as urea, ammonia, and uric acid nitrogen in most of the salivas examined led us to subject our experimental procedure to careful critical study in order to determine whether losses in amino acid nitrogen by precipitation or otherwise has occurred. Morris and Jersey (3) have reported considerable amounts of amino acid nitrogen in saliva and noted that the quantity present in saliva obtained after paraffin chewing was greater than that in saliva from the resting glands. We were unable to remove the ammonia from our filtrates by permutit and were accordingly unable to utilize the Folin method for amino acids. Morris and Jersey state that the application of the Folin-Wu methods to saliva proved entirely satisfactory, but do not give details as to how the amino acid method was applied. The method of removal of protein used by them has not, in our hands,

² Attention should be called to the fact that the highest residual non-protein nitrogen figure observed (Subject T. T., Table III) was obtained from a saliva which contained a sufficient amount of blood to permit of the recognition of its presence by the faint color. Since blood contains a much greater amount of residual non-protein nitrogen than saliva, this unusually high value for residual non-protein nitrogen in the saliva is presumably due, to some extent, at least, to the presence of blood. In most other cases in which high figures for saliva were obtained, the salivas were collected on warm days and stood for an hour or more before precipitation of the protein so that autolysis between the time of collection and of deproteinization probably accounts for the high residual non-protein nitrogen. Our decomposition experiments with saliva have shown that this fraction of nitrogen increases if salivas are allowed to stand and its formation is not checked as is the ammonia formation by the presence of a preservative (chloroform).

yielded a filtrate from which the considerable amounts of ammonia present could be removed by permutit prior to the amino acid determination. It seemed possible that the method of precipitation developed by us might precipitate also the greater part of the amino acids. A solution of alanine, glycine, and aspartic acid was prepared containing 300 mg. of nitrogen per liter, 100 mg. of nitrogen being derived from each of the three acids. Known amounts of this amino acid mixture were added to saliva and the non-protein nitrogen was determined in the filtrates from the original saliva and from the saliva to which the amino acid mixture had been added. Casein was digested by trypsin, and further hydrolyzed by boiling with hydrochloric acid until the biuret reaction was negative. This mixture of amino acids was added to saliva

TABLE VI.

Source of amino acids added.	Nitrogen added as amino acids.	Salivary non-protein nitrogen found.	Extra nitrogen recovered.
	<i>mg.</i>	<i>mg.</i>	<i>mg.</i>
Pure glycine, alanine, and aspartic acid.	0.0 12.0	12.54 24.86	12.32
Tryptic digest of casein completely hydrolyzed.....	0.0 10.0	9.41 18.31	8.90

and the non-protein nitrogen content of the filtrates determined as in the previous experiments. Typical data are recorded in Table VI. In all cases, the added nitrogen was satisfactorily recovered, thus demonstrating that under the conditions with which we were concerned, significant amounts of nitrogen were not precipitated. Losses of amino acids in our method of deproteinization can hardly explain the discrepancy between our figures for "residual" non-protein nitrogen and those of Morris and Jersey for amino acid nitrogen.

We also carried out a series of experiments in which the Folin amino acid procedure was used with the salivary filtrates without any attempt to remove the ammonia. Both glycine and ammonium sulfate were used as standards for comparison with the unknowns. In every case, the color of the unknown was pink,

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